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3092

Morphology of Various Strains of the Trypanosome causing Disease in Man in Nyasaland.—The Mzimba Strain.

BY SURGEON-GENERAL SIR DAVID BRUCE, C.B., F.R.S., A.M.S.; MAJORS DAVID HARVEY AND A. E. HAMERTON, D.S.O., R.A.M.C.; AND LADY BRUCE, R.R.C.

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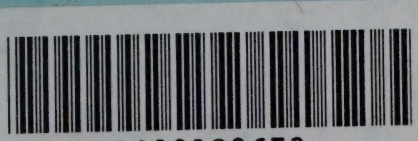

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Morphology of Various Strains of the Trypanosome causing Disease in Man in Nyasaland.—The Mzimba Strain.

By Surgeon-General Sir DAVID BRUCE, C.B., F.R.S., A.M.S.; Majors DAVID HARVEY and A. E. HAMERTON, D.S.O., R.A.M.C.; and Lady BRUCE, R.R.C.

(Scientific Commission of the Royal Society, Nyasaland, 1913.)

(Received May 5,—Read May 29, 1913.)

[PLATES 1–3.]

INTRODUCTION.

Up to the present time it has usually been considered that almost all the cases of Human trypanosome disease in man in Nyasaland have been confined to a small area. This, the so-called Sleeping-Sickness District, has been described in a former paper,* but it may be repeated here that it is the part of the "fly-country" lying along the western shore of Lake Nyasa,

* 'Roy. Soc. Proc.,' B, vol. 86, p. 274.

between S. lat. 13° 20′ and 13° 50′, and extending some twenty miles inland. Through the centre of this area a road runs from Domira Bay on the Lake into North-East Rhodesia. This road, until lately, was a principal highway between the coast and Central Africa. Dr. Aylmer May, the Principal Medical Officer of North-East Rhodesia, who lately visited Kasu, informed the Commission that it was along this trade-route that all the North-East Rhodesian cases of Human trypanosome disease have occurred. It is said that some 25,000 native porters passed along this road every year, and as they entered a *Glossina palpalis* area at the Congo end of their journey, it seemed at first natural to suspect that the disease was true Sleeping Sickness, and had spread from west to east along this trade-route. This suspicion was shown to be groundless by the discovery that the parasite causing the disease in North-East Rhodesia and Nyasaland is not *Trypanosoma gambiense*, but a distinct and separate species giving rise to a totally different disease. The question then arose as to whether this was an imported or indigenous disease. It has, therefore, been one of the objects of this Commission to determine whether the trypanosome causing Human trypanosome disease in Nyasaland is restricted to the game and "fly" of the Proclaimed Area, or if it extends to the north and south along the "fly-belt." If it is found to extend over all the "fly-area" in Nyasaland, then the disease is probably native to the soil and not an importation from Tanganyika or the Congo.

But it will be well at this point to lay down definitely the various opinions or theories at issue. These are three in number.

First, that the Human trypanosome disease of North-East Rhodesia and Nyasaland is caused by a specific trypanosome, *T. rhodesiense*, that the wild game and "fly" are heavily infected with it, and that *T. brucei*, or Nagana, is absent altogether. This is the theory held by one school.

Second, that the wild game and "fly" are heavily infected throughout these "fly-areas" by *T. brucei*, but that at certain places or foci another trypanosome, *T. rhodesiense*, occurs, which is pathogenic to man as well as the other animals. That these two species of trypanosomes are indistinguishable morphologically or by their action on animals, except that one is capable of infecting man and the other not. That the only way to separate them is by inoculating man: if the man reacts it is *T. rhodesiense*, if not, *T. brucei*.

Third, that *T. brucei*—a common trypanosome of wild game, whose distribution extends from Zululand to the Sudan—and *T. rhodesiense* are one and the same species of trypanosome, and that wherever wild game and *G. morsitans* are found there also will be found cases of trypanosome

disease in man. That the cause of the sparsity of cases in man in these areas is due to the fact that man is more or less refractory to the trypanosome, and that it is only rarely that the "fly" meets with a susceptible subject. That this is the reason why the cases of Human trypanosome disease in the Luangwa valley and in Nyasaland do not tend to increase in numbers. The disease remains stationary, as it probably has done during the last thousand years. This is the working hypothesis held at present by this Commission.

These, then, are the points at issue, and it would appear at present that the only way of solving the problem will be by searching and finding out whether cases of *T. brucei* disease, or Nagana, in man occur wherever *G. morsitans* and this parasite are found together. Already cases have been found on the Rovuma river on the borders of German and Portuguese East Africa, and in the Hartley District south of the Zambesi, in Europeans and natives, who certainly could only have contracted the disease in these widely separated districts.

Thanks to Mr. Garden, the Government Veterinary Officer, the Commission have had the opportunity of studying a trypanosome of the Nagana type found in the blood of a donkey at Mzimba (lat. 11° 55′ S., long. 33° 35′ E.), about 100 miles north of the northern border of the Proclaimed Area. It is proposed, then, in this paper to describe this trypanosome, in pursuance of the policy of describing in detail as many strains as possible of this type of trypanosome, if peradventure some method of separating *T. brucei* from *T. rhodesiense* may be discovered, or of proving them to be one and the same species.

Morphology of the Mzimba Strain.

A. *Living, Unstained.*

The movements of this trypanosome in the living condition are similar to *T. brucei* and *T. gambiense* in being non-translatory.

B. *Fixed and Stained.*

The blood films were fixed, stained and measured as previously described in the "Proceedings."*

* 'Roy. Soc. Proc.,' B, vol. 81, pp. 16 and 17.

Table I.—Measurements of the Length of the Trypanosome of the Mzimba Strain.

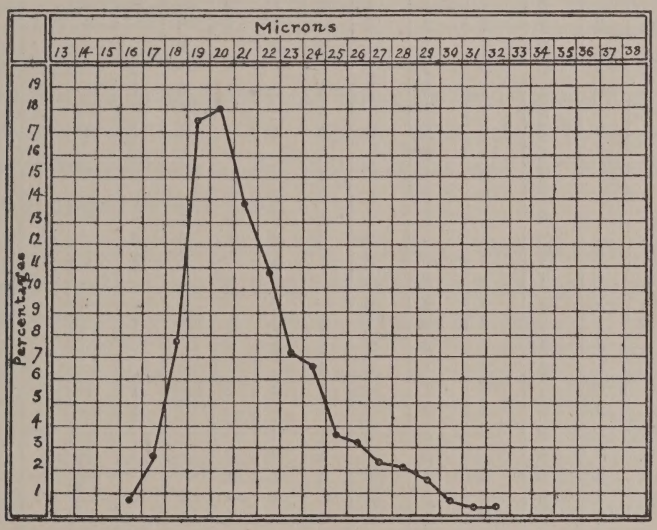
Date.	No. of expt.	Animal.	Method of fixing.	Method of staining.	In microns.		
					Average length.	Maximum length.	Minimum length.
1912.							
Mar. 28.....	365	Donkey	Osmic acid	Giemsa	18·3	21·0	16·0
,, 2.....	384	Goat	,,	,,	19·8	26·0	18·0
,, 27.....	368	,, ...	,,	,,	20·1	23·0	17·0
Apr. 22.....	381	,, ...	,,	,,	20·2	25·0	18·0
,, 29.....	384	,, ...	,,	,,	22·8	32·0	18·0
,, 29.....	402	Monkey	,,	,,	21·2	30·0	17·0
May 6.....	402	,, ...	,,	,,	21·6	29·0	18·0
Mar. 27.....	366	Dog	,,	,,	22·7	30·0	19·0
,, 27.....	367	,, ...	,,	,,	22·2	29·0	19·0
,, 28.....	366	,, ...	,,	,,	22·0	30·0	19·0
,, 28.....	367	,, ...	,,	,,	21·6	30·0	19·0
,, 29.....	366	,, ...	,,	,,	22·0	32·0	17·0
,, 29.....	367	,, ...	,,	,,	20·9	24·0	19·0
,, 30.....	366	,, ...	,,	,,	22·3	29·0	17·0
Apr. 1.....	366	,, ...	,,	,,	20·2	24·0	17·0
,, 4.....	366	,, ...	,,	,,	20·9	25·0	16·0
,, 4.....	366	,, ...	,,	,,	21·6	26·0	17·0
,, 8.....	366	,, ...	,,	,,	21·8	31·0	19·0
,, 8.....	366	,, ...	,,	,,	20·3	27·0	17·0
,, 8.....	387	,, ...	,,	,,	23·1	29·0	17·0
,, 8.....	388	,, ...	,,	,,	19·6	23·0	18·0
,, 11.....	366	,, ...	,,	,,	22·0	31·0	18·0
,, 11.....	388	,, ...	,,	,,	19·6	21·0	18·0
,, 15.....	387	,, ...	,,	,,	22·0	30·0	18·0
,, 15.....	388	,, ...	,,	,,	20·6	25·0	16·0
May 9.....	512	Rat	,,	,,	23·3	31·0	19·0
,, 9.....	512	,, ...	,,	,,	24·3	29·0	19·0
,, 9.....	513	,, ...	,,	,,	22·6	28·0	16·0
,, 9.....	513	,, ...	,,	,,	21·8	26·0	16·0
,, 10.....	512	,, ...	,,	,,	21·2	26·0	19·0
,, 10.....	512	,, ...	,,	,,	21·1	27·0	18·0
,, 10.....	513	,, ...	,,	,,	20·9	25·0	18·0
,, 10.....	513	,, ...	,,	,,	20·3	23·0	17·0
,, 11.....	512	,, ...	,,	,,	22·1	28·0	18·0
,, 11.....	512	,, ...	,,	,,	21·0	27·0	18·0
,, 11.....	513	,, ...	,,	,,	20·2	24·0	17·0
,, 11.....	513	,, ...	,,	,,	22·2	30·0	18·0
,, 13.....	512	,, ...	,,	,,	20·4	25·0	18·0
,, 13.....	512	,, ...	,,	,,	20·2	26·0	18·0
,, 13.....	513	,, ...	,,	,,	19·4	26·0	17·0
,, 13.....	513	,, ...	,,	,,	19·3	23·0	17·0
,, 14.....	512	,, ...	,,	,,	21·7	28·0	17·0
,, 14.....	512	,, ...	,,	,,	21·5	28·0	17·0
,, 14.....	513	,, ...	,,	,,	21·7	28·0	19·0
,, 14.....	513	,, ...	,,	,,	22·5	29·0	19·0
,, 15.....	512	,, ...	,,	,,	24·1	30·0	17·0
,, 15.....	512	,, ...	,,	,,	24·5	32·0	18·0
,, 15.....	513	,, ...	,,	,,	21·2	26·0	19·0
,, 15.....	513	,, ...	,,	,,	21·6	28·0	18·0
,, 16.....	513	,, ...	,,	,,	21·2	31·0	18·0
					21·4	32·0	16·0

The average length of the trypanosome of the Mzimba strain, in different species of animals, taken from Table I, is as follows :—

Table II.

Species of animal.	Number of trypanosomes measured.	In microns.		
		Average length.	Maximum length.	Minimum length.
Donkey.....	20	18·3	21·0	16·0
Goat.....	80	20·7	32·0	17·0
Monkey	40	21·4	30·0	17·0
Dog	360	21·4	32·0	16·0
Rat	500	21·6	32·0	16·0

CHART 1.—Curve representing the Distribution, by Percentages, in respect to Length, of 1000 Individuals of the Trypanosome of the Mzimba Strain.

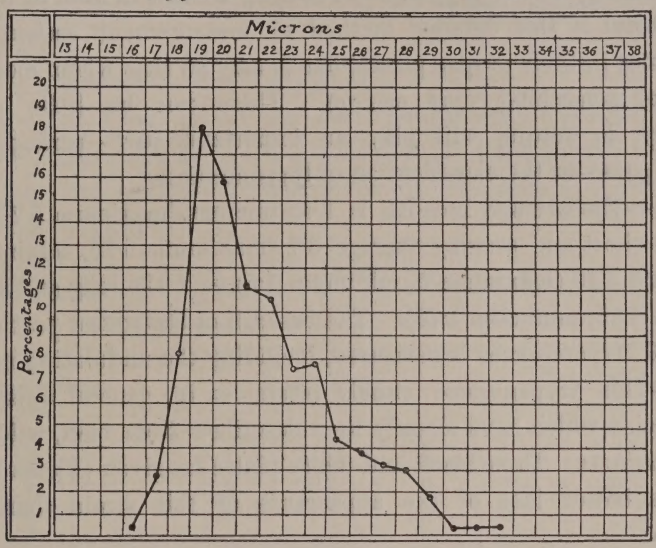


This curve is made up of measurements from 20 specimens of trypanosomes taken from the original donkey, 80 from the goat, 40 from the monkey, 360 from the dog, and 500 from the rat.

Table III.—Distribution in respect to Length of 1000 Individuals of the Trypanosome of the Mzimba Strain.

Animal.	In microns.																Average length.
	16.	17.	18.	19.	20.	21.	22.	23.	24.	25.	26.	27.	28.	29.	30.	31.	32.	
Donkey	3	1	6	7	2	1	—	—	—	—	—	—	—	—	—	—	—	18·3
Goat	—	—	5	6	4	2	1	1	—	—	1	—	—	—	—	—	—	19·8
,,	—	1	—	5	8	3	2	1	—	—	—	—	—	—	—	—	—	20·1
,,	—	—	4	5	4	2	2	1	1	1	—	—	—	—	—	—	—	20·2
,,	—	—	1	2	4	2	2	2	1	3	—	—	1	1	—	—	1	22·8
Monkey	—	3	4	1	4	2	—	1	1	—	—	1	1	1	1	—	—	21·2
,,	—	—	1	2	6	4	2	1	2	—	—	—	1	1	—	—	—	21·6
Dog	—	—	—	1	3	6	4	1	1	—	—	1	1	1	1	—	—	22·7
,,	—	—	—	1	1	9	3	2	1	1	—	1	—	1	—	—	—	22·2
,,	—	—	—	1	4	5	3	5	—	1	—	—	—	—	1	—	—	22·0
,,	—	—	—	5	1	5	4	2	2	—	—	—	—	—	1	—	—	21·6
,,	—	1	—	1	5	6	2	1	—	—	2	1	—	—	—	—	1	22·0
,,	—	—	—	3	3	8	5	—	1	—	—	—	—	—	—	—	—	20·9
,,	—	1	—	2	3	2	2	2	5	1	1	—	—	1	—	—	—	22·3
,,	—	2	—	6	4	3	3	1	1	—	—	—	—	—	—	—	—	20·2
,,	2	1	—	3	1	4	4	2	1	2	—	—	—	—	—	—	—	20·9
,,	—	1	2	1	4	2	4	—	2	1	3	—	—	—	—	—	—	21·6
,,	—	—	—	5	4	4	—	2	2	—	2	—	—	—	—	1	—	21·8
,,	—	1	4	6	3	1	2	—	—	1	—	2	—	—	—	—	—	20·3
,,	—	1	—	2	1	3	2	2	2	1	3	1	1	1	—	—	—	23·1
,,	—	—	4	6	7	1	—	2	—	—	—	—	—	—	—	—	—	19·6
,,	—	—	2	5	2	2	2	1	1	1	1	1	1	—	—	1	—	22·0
,,	—	—	3	4	11	2	—	—	—	—	—	—	—	—	—	—	—	19·6
,,	—	—	1	1	6	1	4	4	1	—	—	—	1	—	1	—	—	22·0
,,	1	—	1	3	6	3	3	—	2	1	—	—	—	—	—	—	—	20·6
Rat	—	—	—	2	1	5	1	1	2	4	2	—	1	—	—	1	—	23·3
,,	—	—	—	3	1	—	2	2	2	1	3	3	—	3	—	—	—	24·3
,,	1	1	1	1	2	—	3	1	5	—	2	2	1	—	—	—	—	22·6
,,	1	2	1	1	2	2	3	—	1	5	2	—	—	—	—	—	—	21·8
,,	—	—	—	4	5	2	6	—	2	—	1	—	—	—	—	—	—	21·2
,,	—	—	1	4	6	3	1	1	2	1	—	1	—	—	—	—	—	21·1
,,	—	—	2	1	7	3	3	2	1	1	—	—	—	—	—	—	—	20·9
,,	—	1	1	8	2	1	3	4	—	—	—	—	—	—	—	—	—	20·3
,,	—	—	2	—	6	1	3	1	3	2	—	1	1	—	—	—	—	22·1
,,	—	—	2	2	4	7	—	3	1	—	—	1	—	—	—	—	—	21·0
,,	—	2	3	3	5	1	2	1	3	—	—	—	—	—	—	—	—	20·2
,,	—	—	2	4	—	6	1	1	1	1	—	2	1	—	1	—	—	22·2
,,	—	—	3	5	5	1	2	2	1	1	—	—	—	—	—	—	—	20·4
,,	—	—	2	9	2	3	2	—	1	—	1	—	—	—	—	—	—	20·2
,,	—	2	3	9	3	1	1	—	—	—	1	—	—	—	—	—	—	19·4
,,	—	1	8	4	2	2	1	2	—	—	—	—	—	—	—	—	—	19·3
,,	—	1	1	3	2	2	3	3	3	1	—	—	1	—	—	—	—	21·7
,,	—	3	2	3	2	1	2	1	2	—	—	2	2	—	—	—	—	21·5
,,	—	—	—	6	4	—	4	1	2	—	1	1	1	—	—	—	—	21·7
,,	—	—	—	4	2	2	4	3	1	—	1	—	1	2	—	—	—	22·5
,,	—	1	2	1	1	—	2	1	2	2	1	1	3	2	1	—	—	24·1
,,	—	—	2	1	1	1	1	3	1	2	1	2	1	2	—	—	2	24·5
,,	—	—	—	7	1	5	1	3	1	—	2	—	—	—	—	—	—	21·2
,,	—	—	1	2	8	3	2	—	1	1	1	—	1	—	—	—	—	21·6
,,	—	—	2	4	5	4	—	2	1	—	—	—	1	—	—	1	—	21·2
Total	8	27	79	175	180	139	109	72	66	36	32	24	22	16	7	4	4	
Percentages ...	0·8	2·7	7·9	17·5	18·0	13·9	10·9	7·2	6·6	3·6	3·2	2·4	2·2	1·6	0·7	0·4	0·4	

CHART 2.—Curve representing the Distribution, by Percentages, in respect to Length, of 500 Individuals of the Trypanosome of the Mzimba Strain, taken from Rats alone.



The similarity in the curve of the Mzimba strain and the curve of the Wild-game strain* is remarkable, and there can be little doubt that the same trypanosome is being dealt with. This is what might be expected, seeing there is probably only one animal between the wild *G. morsitans* and the experimental animal in both cases.

Table IV.—Measurements giving the Average Distance from the Posterior Extremity to Micronucleus, Micronucleus to Nucleus, etc., of 1000 Individuals of the Trypanosome of the Mzimba Strain.

Posterior extremity to micronucleus.	Micronucleus to nucleus.	Diameter of nucleus.†	Nucleus to anterior extremity.	Flagellum.
Short and Stumpy (16–21 microns), 608 Individuals.				
1·2	4·4	2·9	10·6	0·4
Intermediate (22–24 microns), 247 Individuals.				
1·5	5·3	3·0	10·4	2·6
Long and Slender (25–32 microns), 145 Individuals.				
1·8	5·8	3·0	10·9	5·5
Average 1·5	5·2	3·0	10·6	2·8

† These measurements are made along the long axis of the trypanosome, and therefore if an oval nucleus is lying transversely, the measurement given will not represent the greatest length of the nucleus.

* 'Roy. Soc. Proc.,' B, vol. 86, p. 394.

Table IV agrees very closely with the same table given in the paper on the "Morphology of the Trypanosome causing Disease in Man in Nyasaland."* It is doubtful if this detailed method of measurement assists in putting trypanosomes in their proper places, but it may be that when more work has been done, something may emerge. There can be little doubt that morphology, in future, will play an important part—perhaps the most important part—in the classification of trypanosomes.

Breadth.—The average breadth is 3·45 microns (maximum 5·75, minimum 1·5). The short and stumpy average 3·7 (maximum 5·75, minimum 2), the intermediate 3·16 (maximum 5, minimum 1·5), and the long and slender 2·84 (maximum 4·25, minimum 1·75). The breadth of the widest part of the body of the 1000 trypanosomes is measured, including the undulating membrane.

Shape.—This trypanosome closely resembles in general appearance the various strains which have already been described from man, wild game and wild *G. morsitans.* Three black-and-white plates are given (Plates 1–3), which will give a better idea of the appearance of this strain than a written description.

Table V.—Percentage of Posterior-Nuclear Forms found among the Short and Stumpy Varieties of the Trypanosome of the Mzimba Strain.

Date.	Experiment No.	Animal.	Percentage among short and stumpy forms.	Proportion to all forms per 1000.
1912.				
Mar. 27 ...	367	Dog	0	0
„ 28 ...	367	„	10	60
„ 28 ...	366	„	3	18
„ 29 ...	367	„	8	48
„ 29 ...	366	„	13	78
„ 30 ...	366	„	12	72
Apr. 1 ...	366	„	7	42
„ 25 ...	387	„	15	90
„ 29 ...	387	„	25	150
May 2 ...	387	„	37	222
„ 10 ...	513	Rat	11	66
„ 10 ...	512	„	22	132
„ 11 ...	513	„	19	114
„ 11 ...	512	„	12	72
„ 13 ...	513	„	7	42
„ 13 ...	512	„	5	30
„ 14 ...	513	„	10	60
„ 15 ...	513	„	28	168
„ 16 ...	513	„	33	198
„ 16 ...	512	„	3	18
		Average	14·7	88

* 'Roy. Soc. Proc.,' 1912, B, vol. 85, p. 428.

Micronucleus.—Situated, on an average, 1·8 microns from the posterior extremity in the long and slender, 1·5 in the intermediate, and 1·2 in the short and stumpy.

Undulating Membrane.—Well developed.

Flagellum.—The flagellum in the short and stumpy averages 0·4 micron (maximum 4, minimum 0·2), in the intermediate 2·6 (maximum 6, minimum 1) and in the long and slender 5·5 (maximum 10, minimum 2). Total average 2·8 (maximum 10, minimum 1).

Table VI.—Number of Flagellated and Non-flagellated Forms found among 1000 Trypanosomes of the Mzimba Strain.

Short and stumpy.			Intermediate.			Long and slender.		
Length, microns.	Non-flagellated.	Flagellated.	Length, microns.	Non-flagellated.	Flagellated.	Length, microns.	Non-flagellated.	Flagellated.
16	8	0	22	34	75	25	0	36
17	27	0	23	12	60	26	0	32
18	73	6	24	5	61	27	0	24
19	154	21				28	0	22
20	153	27				29	0	16
21	94	45				30	0	7
						31	0	4
						32	0	4
Totals	509	99		51	196		0	145

If the trypanosomes are divided into non-flagellar and flagellar, there are 56 per cent. of the former and 44 per cent. of the latter.

If we divide the 1000 Mzimba trypanosomes by length into short and stumpy (16 to 21 microns), intermediate (22 to 24 microns), and long and slender (25 to 32 microns), the percentages are 60·8, 24·7, and 14·4 respectively.

Conclusions.

1. The trypanosome of the Mzimba strain is the same species as that occurring in the wild game inhabiting the Proclaimed Area, Nyasaland.

2. It has already been concluded that this species is *T. brucei* vel *rhodesiense.*

3. Hence it would appear that wild *G. morsitans* occurring in a district 100 miles north of the Proclaimed Area are infected with the trypanosome which causes the Human trypanosome disease of Nyasaland.

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DESCRIPTION OF PLATES.

Plate 1.

T. brucei vel *rhodesiense.* Short and stumpy forms, 16-21 microns in length. Stained Giemsa. × 2000.

Plate 2.

T. brucei vel *rhodesiense.* Intermediate forms, 22–24 microns in length. Stained Giemsa. × 2000.

Plate 3.

T. brucei vel *rhodesiense.* Long and slender forms, 25–32 microns in length. Stained Giemsa. × 2000.

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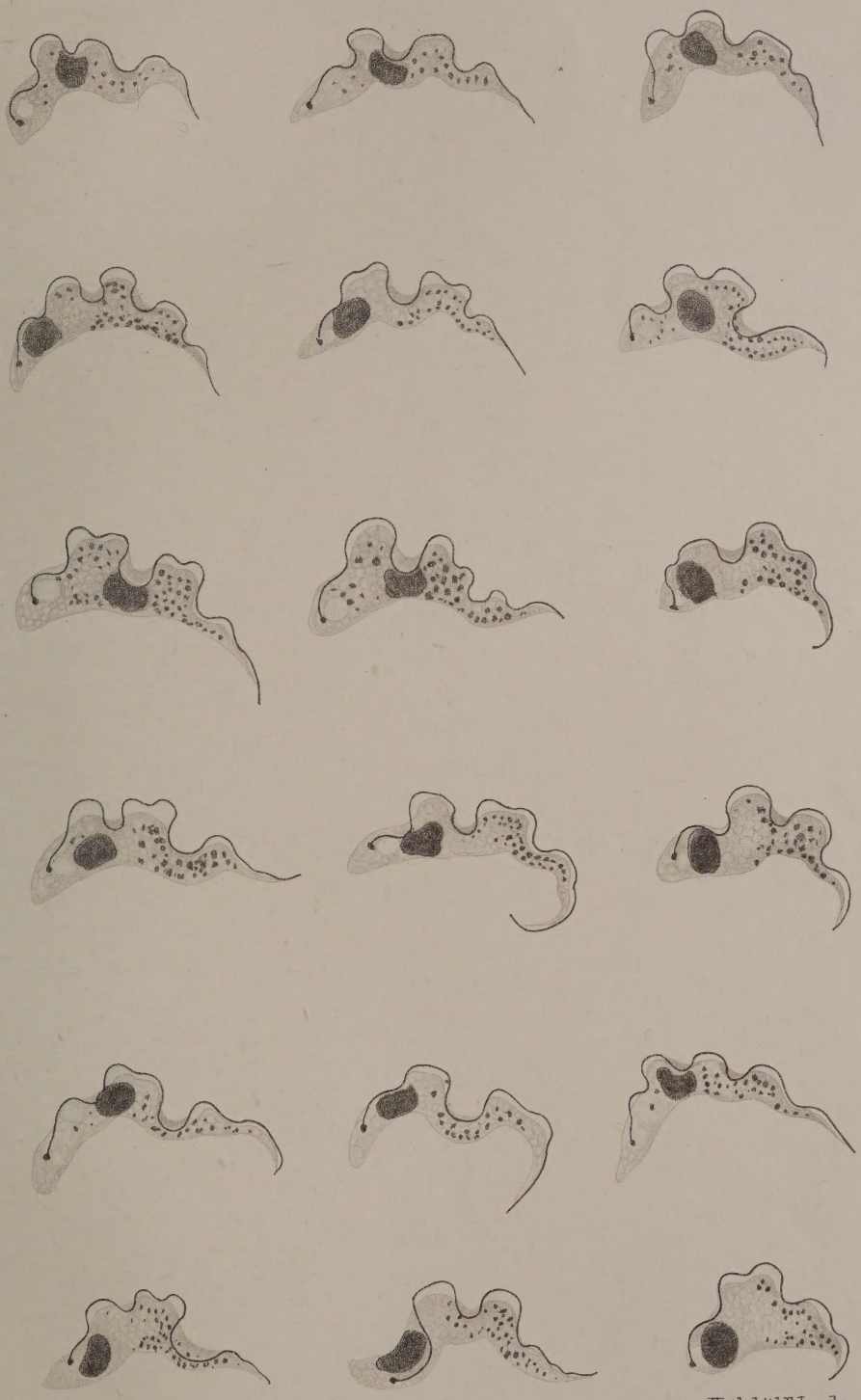

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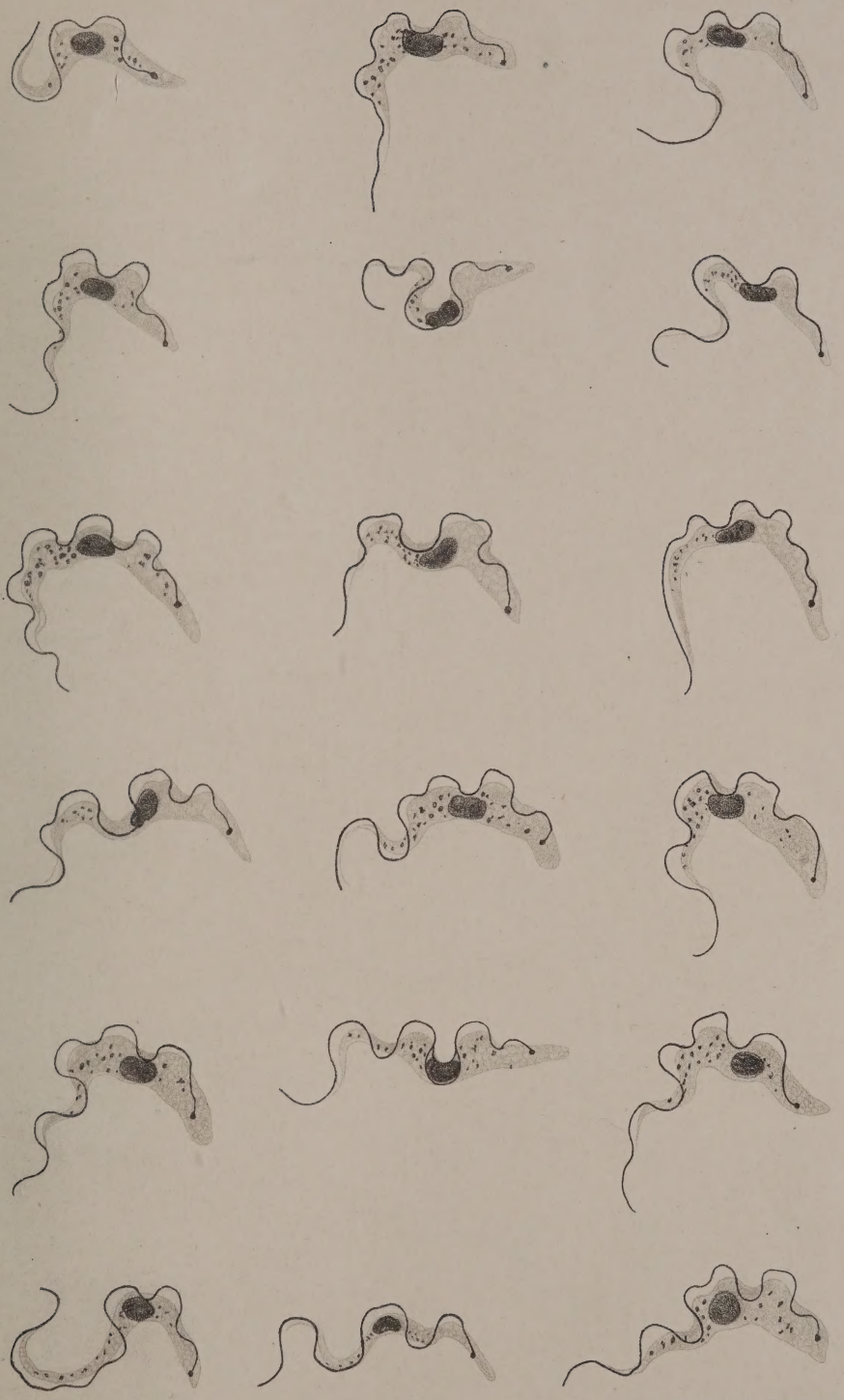
Hüth, lith^r London.

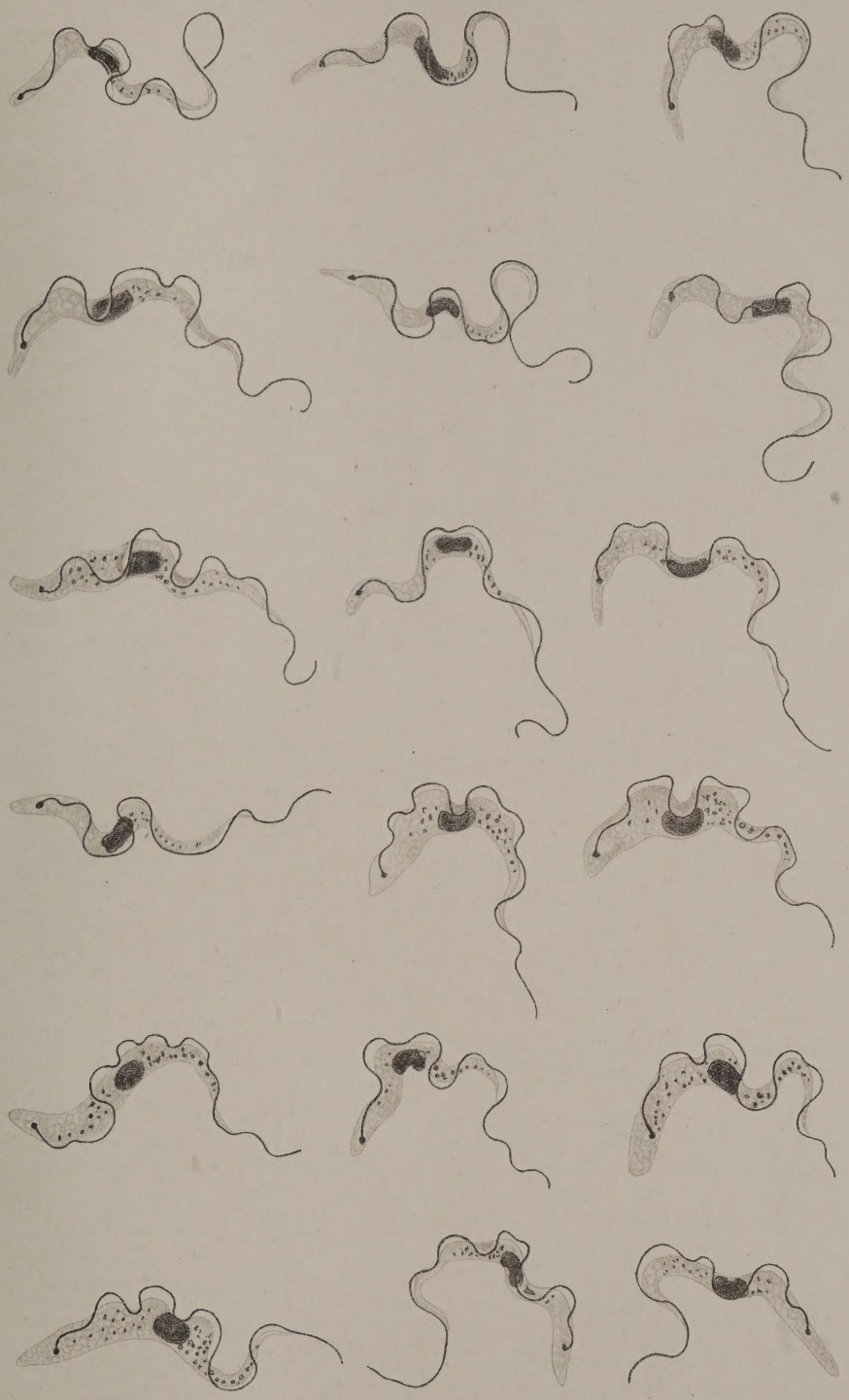
Short & Stumpy



M.E. Bruce del.

Huth, Lith.r London.

Intermediate



M. E. Bruce, del. Huth Lith^f London.

Long & Slender